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13. ABSTRACT (Maximum 200 words)

We have utilized germline mutagenesis to dissect innate immune sensing and effector pathways, and have made a number of important findings related to the mechanism of TLR signal transduction. In particular, we were able to solve the molecular basis of MyD88-independent signaling, and to determine the cause of adjuvanticity (signaling via Trif and the production of type I interferons). Finally, our work has led to the first realization of the importance of the TLR9 -> MyD88 signaling axis and the TLR3 -> Trif signaling axis in responses to MCMV. These discoveries have important implications for the further study of innate resistance to both viral and bacterial infections, for the development of vaccines, and for the design of molecular sensors of infection.

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FINAL REPORT

Performer: Bruce Beutler, MD
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Project Name: Direct detection of microbial infection through activation coupling of the Toll-like receptors

During the years of funding provided by DARPA ("Direct detection of microbial infection through activation coupling of the Toll-like receptors"), we established a means of detecting novel innate immunodeficiency mutations, some of which have enhanced understanding of how infectious stimuli such as Gram-negative bacteria (or LPS) and also viruses such as (especially viruses of the herpes virus family) trigger an immune response. In all, we have identified a total of 33 innate immunodeficiency mutations, have mapped ten of these mutations to chromosomes, and have positionally cloned eight of them.

Our project began as an effort aimed at using Toll-like receptors, the primary mammalian sensors of bacterial infection, as a means of constructing an early warning system by which a detectable signal could be generated. The project initially encountered difficulties when attempts were made to construct reporters based on the human Toll-like receptor 4 (TLR4) molecules, in the absence of complete information concerning the structure of this protein complex and its adapters. Using a forward genetic strategy, in which germline mutations were introduced with the alkylating agent ENU, we attempted to better understand the structure of the receptor and its transducer molecules. We identified a novel mutation called *Lps2*, and positionally cloned a previously unknown adapter molecule for TLR4, now termed TRIF. We also inferred the existence of a second adapter called adapter X (now widely known as TRAM).

Our work has brought the total number of adapters that serve TLR4 to four, and has laid the groundwork for what might be a more successful attempt at utilizing this TLR4 and other TLRs as universal microbial sensors. While it was previously unappreciated, TLR4 does not directly engage MyD88, and this was the basis of the detection system that we had attempted to construct. Rather, TLR4 appears to engage TRAM and MAL. A detection system based on the use of these proteins might prove more successful than one based on the use of MyD88.

We also identified mutations in the *Tnfa* gene, in the *Tlr9* gene, in the *Cd36* gene, the *Scd1* (stearoyl-coA desaturase) gene, and in the *Lyst* gene as innate immunodeficiency lesions. A total of 15 other mutations that create hypersusceptibility to infection by MCMV (a mouse equivalent of human cytomegalovirus), and eight mutations that create susceptibility to *Listeria monocytogenes* have been identified as well. These mutations are a priceless resource for further investigation of how innate immune sensing and effector function work.

Among our accomplishments, we have also shown that not only bacteria but also viruses are sensed by the Toll-like receptors. This is important information, in the sense that previously, the pathway by which viral stimuli such as unmethylated DNA and double stranded RNA could evoke a signal were unknown. It is now clear that both the Tlr3→TRIF pathway and the Tlr9→MyD88 pathway are absolutely required for effective containment of MCMV infection. By implication, human viral defense pathways also depend upon these molecules.

To summarize our achievements, it could be said that we were not successful of the primary goal of designing a direct-coupled system involving the Toll-like receptors for the detection of microbial pathogens. However, we were highly successful in elucidating many of the most important components used by innate immune system to detect infection, and the basic information so adduced will lead to advances in many areas of infection and inflammation biology.